

CLAIMS

1. A method for diagnosis/prognosis of breast cancer comprising the following stages:

5 A - the nuclear material is extracted from a biological specimen,
 B - at least one pair of amplification primers is used for obtaining amplicons
 of at least one target sequence of the nuclear material
 C - at least one detection probe is used for detecting the presence of said
 amplicons

10 characterized in that, in stage B, said pair of primers comprises at least one
 amplification primer comprising at least 10 nucleotide motifs of a nucleotide
 sequence selected from SEQ ID No. 1 to SEQ ID No. 20 and/or in stage C, said
 detection probe comprises at least 10 nucleotide motifs of a nucleotide sequence
 selected from SEQ ID No. 1 to SEQ ID No. 20.

- 15 2. The method for diagnosis/prognosis of breast cancer as claimed in claim 1,
 characterized in that, in stage B, said pair of primers is selected from the
 following pairs of primers:

- 20 □ a first amplification primer comprising at least 10 nucleotide motifs of
 nucleotide sequence SEQ ID No. 1 and a second amplification primer
 comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 2;
 □ a first amplification primer comprising at least 10 nucleotide motifs of
 nucleotide sequence SEQ ID No. 3 and a second amplification primer
 comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 4;
 25 □ a first amplification primer comprising at least 10 nucleotide motifs of
 nucleotide sequence SEQ ID No. 5 and a second amplification primer
 comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 6;
 □ a first amplification primer comprising at least 10 nucleotide motifs of
 nucleotide sequence SEQ ID No. 7 and a second amplification primer
 30 comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 8;

- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 13 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 14;
 - 5 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 15 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 16;
 - a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 17 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 18;
 - 10 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 19 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 20.
3. The method for diagnosis/prognosis of breast cancer as claimed in either one of
- 15 claims 1 or 2 in which said pair of primers comprises at least one amplification primer comprising a promoter permitting the initiation of transcription by a polymerase of bacteriophage T7.
4. The method for diagnosis/prognosis of breast cancer as claimed in any one of
- 20 claims 1 to 3 in which, in stage C, the detection probe comprises a fluorophore and a quencher.
5. The method as claimed in any one of claims 1 to 4 in which the target sequence comprises a gene selected from ESR1, ESR2, PGR, HER2.
- 25 6. The method as claimed in any one of claims 1 to 5 in which stages B and C are carried out simultaneously.
7. The method as claimed in any one of claims 1 to 6, characterized in that, in stage
- 30 B, at least one pair of amplification primers is used additionally, for obtaining amplicons specific to a housekeeping gene.

8. The method as claimed in claim 7, characterized in that the amplification primer for obtaining amplicons specific to a housekeeping gene comprises at least 10 nucleotide motifs of a sequence selected from SEQ ID No. 25 to 29.

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9. The method as claimed in claim 7, characterized in that said pair of amplification primers for obtaining amplicons specific to a housekeeping gene is selected from the following pairs of primers:

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- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 27 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 28;
- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 25 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 26.

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10. An amplification primer comprising at least 10 nucleotide motifs of a nucleotide sequence selected from SEQ ID No. 1 to SEQ ID No. 20; 25 to 29.

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11. The amplification primer as claimed in claim 10, comprising a promoter permitting the initiation of transcription by a polymerase of bacteriophage T7.

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12. A pair of amplification primers selected from the following pairs of primers:

- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 1 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 2;
- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 3 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 4;

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- a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 5 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 6;
- 5 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 7 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 8;
- 10 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 13 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 14;
- 15 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 15 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 16;
- 20 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 17 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 18;
- 25 □ a first amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 19 and a second amplification primer comprising at least 10 nucleotide motifs of nucleotide sequence SEQ ID No. 20.

13. The pair of primers as claimed in claim 12, in which said first primer comprises a promoter permitting the initiation of transcription by a polymerase of bacteriophage T7.

14. The use of at least one amplification primer as claimed in claim 10 or 11 and/or of a pair of primers as claimed in claim 12 or 13 in a NASBA amplification reaction.
- 5 15. A detection probe comprising at least 10 nucleotide motifs of a nucleotide sequence selected from SEQ ID No. 1 to SEQ ID No. 20.
16. The detection probe as claimed in claim 15, comprising a fluorophore and a quencher.
- 10 17. The use of at least one primer as claimed in claim 10 or 11 and/or at least one pair of primers as claimed in claim 12 or 13 and/or at least one detection probe as claimed in claim 15 or 16 for diagnosis/prognosis of breast cancer.
- 15 18. A kit for diagnosis/prognosis of breast cancer comprising at least one primer as claimed in claim 10 or 11 and/or at least one pair of primers as claimed in claim 12 or 13 and/or at least one detection probe as claimed in claim 15 or 16.